θRadegen Bio

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Skunkworks Project Olympus:

R&D Project Proposal: To develop a flow-cell based DNA synthesis platform using an Olympus benchtop fluorescence microscope with Radagen Bio's Theta+ DNA stepwise forging enzymes in a "flow over whole substrate" oligo library synthesis method. This method is conceptualized to be conducted in an Olympus benchtop laser scanning florescence microscope (scanning fluorescence microscopes are prone to freezing, regardless of how costly with enough image capture events). This platform is specifically designed for this task and it is within reason that the company would provide modified software for this task. A modified program is needed since the data acquisition step consumes computer system resources that freeze the computer system) and Microscope slides will be prepared tethered with oligos using Radagen Theta+ forging technology, termed Theta+ scope-substrates. In this method oligos are polymerized by applying continuous flow of a master mix over the **θpAT** substrate containing only 1 dddNTP-**θ***ZaI*. The first step in the process deprotects the first nt of the ssDNA priming substrate by UV light during continuous flow of reaction buffer. Only the spots on the glass slide that contain a growing oligo are illuminated with UV treatment. Next, polymerization master mix flow is applied for 30 seconds followed by a TE wash. Next, the set of oligos requiring polymerization on position 1 of the growing oligos are deprotected with UV light and the polymerization process begins once more. This process produces pools of oligos instead of oligos produced using Radegen Bio's Theta+ DNA forging technology. The number of individual sequences should be similar to that of old-school microarrays. The total fragment length is limited by polymerase efficiency but projected to be a max length of 350 nt. Radegen Bio's theta priming AT oligo (**θpAT)** surface is generated using the original microarray technology developed by Patrick O. Brown. After a google search of microarrays glass slides two classes were observed, 1) modern array glass with highly condensed oligos and 2) old fashioned oligo glass with 2 columns containing 5 23x23 spots of oligos. A recent paper presenting a similar Dtd based method was able to generate enough DNA to store 1MB of binary information, but the synthesis fidelity of individual clusters was poor. The method still has an application since information stored in DNA has executable code up and downstream of the core information that helps transcribe and correct mutations. Using a 23x23 cluster format allows for simple surface preparation methods and makes a ready substrate more affordable if purchased from a supplier. The key however is that clusters of 23x23 with arrays patched in 5 column with 2 cluster patches in each column can be distinguished visually and are far apart from each other to prevent cross-illumination.

> *(The Olympus scanning benchtop microscope is designed to take multiple 3D stacks of images with a potential of surveying a number of x,y points on a slide to capture a picture. The inherit characteristic of this instrument. The **θ**ZaI enzyme was developed using Radegen Bio's rational design framework. This synthetic enzyme was designed based on established strategies for enzyme engineering to have higher efficiency by increasing hydrogen bonding efficiency and reducing reactive residues on the surface of the protein. A reactive residue was strategically placed near the catalytic center to generate an anchoring point for a dNTP.)*

This method will not be used for custom synthetic DNA products using θZaI (Radegen Bio's Dtd enzyme used in the robotic liquid handling-based system). This product is being considered for presenting to Olympus Microscopes for licensing and co-development.